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Short communication

Individual differences in sugar consumption predict amphetamine-induced dopamine overflow in nucleus accumbens

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Abstract

Rats exhibit individual differences in their consumption of sugar and in their response to amphetamine treatments. Intrinsic variation in the functioning of the mesolimbic dopamine system is one potential mechanism underlying the expression of these individual differences. The present experiment examined the relationship between sugar consumption and the dopaminergic response to amphetamine. In vivo microdialysis was used to assess amphetamine-stimulated dopamine overflow in the posterior-medial nucleus accumbens in LOW and HIGH sugar feeders. Sugar consumption correlated significantly with amphetamine-stimulated accumbens-dopamine overflow. HIGH rats exhibited significantly higher levels of amphetamine-stimulated accumbens-dopamine overflow than LOW rats. These results suggest that the propensity to ingest sugar is a predictor of the nucleus accumbens dopaminergic response to amphetamine treatment.

Keywords: Amphetamine; Microdialysis; Dopamine; Individual difference; Nucleus accumbens; (Rat)

1. Introduction

There is a growing body of evidence that rats exhibit individual differences in behaviors thought to be mediated by the mesocorticolimbic dopamine pathway. Animals that exhibit high levels of locomotor activity in a novel environment show a greater locomotor response to amphetamine and cocaine than animals that exhibit low levels of locomotor activity in a novel environment (Piazza et al., 1989; Hooks et al., 1991a). Similarly, rats that consume high amounts of sugar (HIGH) show a greater locomotor response to acute and repeated amphetamine treatments than rats that consume low amounts of sugar (LOW) (Sills and Vaccarino, 1994). Further, rats that show a greater sensitivity to the locomotor activating property of amphetamine more readily self-administer amphetamine (Piazza et al., 1989), indicating an increased sensitivity to the rewarding properties of amphetamine.

Rewarding events, including feeding and self-administration of psychostimulants, are accompanied by activation of the mesolimbic dopamine system (Lyness et al., 1979; Hernandez and Hoebel, 1988). In addition, the locomotor activating effect of amphetamine is dependent on the integrity of this system (Kelly et al., 1975). Recently, Piazza et al. (1991) have shown that rats with enhanced sensitivity to the locomotor activating effect of amphetamine exhibit higher levels of dopamine turnover in the nucleus accumbens than rats with lower sensitivity to the locomotor activating effect of amphetamine, under both baseline conditions and in response to being placed in a novel environment. Rats that show high levels of locomotor activity in a novel environment exhibit higher accumbens-dopamine release than rats with low levels of locomotor activity in a novel environment, under both baseline and cocaine-stimulated conditions (Hooks et al., 1991b).

At present, it is not known whether rats that differ in sugar consumption show differences in accumbens-dopamine release, either under baseline conditions or in response to amphetamine challenges. The present experiment was designed to address this issue. Since HIGH rats show a greater locomotor response to amphetamine (Sills and Vaccarino, 1994) and rats with a high locomotor response to amphetamine exhibit high levels of accumbens-dopamine release under baseline and stimulated conditions (Piazza et al., 1991; Hooks et al., 1991b), the present experiment evaluated the hypothesis that HIGH rats exhibit higher levels of amphetamine-stimulated accumbens-dopamine release than LOW rats.

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2. Materials and method

2.1. Subjects

Male Wistar rats (Charles River, New York) weighing 250–275 g at the start of the experiment were tested for their response to amphetamine. Animals were housed individually in suspended wire mesh cages on a 12 h light/dark cycle (lights on-off at 0600–1800 h). Animals had ad libitum access to Purina lab pellets and water unless otherwise stated. The colony room was maintained at a constant ambient temperature of 21°C. All procedures were approved by the National Institute of Mental Health Animal Care and Use Committee, and followed the NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Feeding phase

For seven consecutive days the normal wire mesh food hoppers containing standard Purina lab pellets (Zeigler Bros., Gardners, PA) were replaced with two $5 \times 5 \times 3$ cm stainless steel containers with pre-weighed amounts of sugar and powdered Purina lab chow (Bio-Serv, Frenchtown, NJ). Animals had access to the test diets for a period of 1 h (2000–2100 h) after which the remaining food (and spillage) was reweighed to determine the amount of food consumed. For the remaining 23 h, animals had ad libitum access to standard Purina lab pellets. On the 8th day, animals were injected intraperitoneally with 0.9% saline, in a volume of 1 ml/kg, immediately prior to presentation of the powdered chow and the granulated sugar. Intake was again measured for 1 h. Subsequently, animals were divided into two groups, LOW and HIGH, based on a median split of their intake of sugar in response to intraperitoneal saline treatment as previously described (Sills and Vaccarino, 1994). Following this initial phase, each animal was implanted with a guide cannula 2 mm dorsal to the posterior-medial nucleus accumbens.

2.3. Surgery

Stereotaxic surgery was conducted under chloral hydrate anesthesia. Rats received unilateral implants of a CMA-10 guide cannula (Carnegie Medicin; Bioanalytical Systems, West Lafayette, IN) at the following stereotaxic co-ordinates: 1.2 mm anterior to bregma, 0.8 mm lateral to bregma, 6.0 mm ventral to the surface of the skull, incisor bar at −3.5 mm (Paxinos and Watson, 1982). Following recovery from surgery, animals were returned to their home cages.

2.4. Microdialysis

At least 1 week following surgery, rats were tested for their dopaminergic response to amphetamine treatment. For purposes of testing, the microdialysis probe (CMA 10, 2.0 mm tip length, Carnegie Medicin, Bioanalytical Systems, West Lafayette, IN) was inserted through the guide cannula into the posterior-medial accumbens and secured in place via a guide cannula assembly the night prior to the test session. The probe was connected to a microinfusion pump (CMA 100, Bioanalytical Systems) and the posterior-medial accumbens was perfused at a rate of 0.3 μl per min continuously for a period of 12–16 h with artificial cerebrospinal fluid (aCSF) consisting of 135 mM sodium chloride, 3.0 mM potassium chloride, 1.2 mM calcium chloride dihydrate, 1.0 mM magnesium chloride, and 2 mM phosphate buffer, pH 6.7. The next morning the rate of perfusion was increased to 1.3 μl/min. Six samples of dialysate were collected prior to the intraperitoneal (i.p.) administration of 1.75 mg/kg amphetamine, after which another nine samples were taken. Baseline dopamine levels were calculated as the average of the last three samples prior to amphetamine treatment. Samples of dialysate were collected every 20 min. All dialysis experiments were conducted between the hours of 1100–1700.

Dialysates were assayed for dopamine using high-pressure liquid chromatography with electrochemical detection (HPLC-EC; ESA Coulochem II electrochemical detector, ESA, MA). The HPLC-EC system was equipped with an HR-80 reverse-phase column (8 cm column packed with 3 μm spherical octadecylsilane), a Model 5020 guard cell, and a Model 5014 analytical cell composed of an amperometric electrode coupled with a coulometric electrode. The potential for the guard cell was set at +0.3 V, with the potential of the first electrode set at −0.125 V and the potential of the second, analytical, electrode set at +0.125 V. The detection limit of this assay was 0.32 pg/sample.

The mobile phase consisted of 75 mM sodium phosphate (NaH_2PO_4), 25 μM ethylenediaminetetraacetic acid (EDTA), 1.5 mM sodium dodecyl sulphate (SDS), 120 ml methanol/liter, and 150 ml acetonitrile/liter, pH 5.6, delivered at a flow rate of 0.6 ml/min. Dialysate samples were injected in a volume of 25 μl. Peak heights of endogenous dopamine were compared to peak heights of a standard solution of 10^{-9} M dopamine (Sigma Chemical Co., St. Louis, MO). The values obtained were corrected for differences in probe recovery between animals; the percent recovery for each probe was determined in vitro prior to implantation. The average percent recovery for the probes used in this study was 10.9% ± 1.4%. There was no difference in percent recovery in probes between LOW and HIGH sugar feeders, $t_{12} = 1.246$, $P > 0.05$.

2.5. Analyses

At the conclusion of testing, each animal was given chloral hydrate overdose. The brain was subsequently removed and 100 μm sections were obtained using a microtome. The sections were stained with thionin and histological verification of cannula and probe tracks was ascertained with light microscopy. Statistical analyses were

carried out using Pearson product moment correlation, Student's t test and repeated measures analysis of variance (ANOVA), followed by post-hoc comparisons using the least significant difference test.

3. Results

As represented in Fig. 1, histological analysis revealed that the microdialysis probes of 14 of 21 rats were correctly placed within the posterior-medial nucleus accumbens (1.0–1.2 mm anterior to bregma). Only the data from these animals were used in all subsequent statistical analyses. An equal number of LOW ($n = 7$) and HIGH ($n = 7$) sugar feeders had probes that were correctly located in the posterior-medial nucleus accumbens.

As shown in Fig. 2A, HIGH rats consumed more sugar than LOW feeders across the seven day period prior to saline treatment, $t_{12} = 2.401$, $P < 0.05$. There was a significant correlation between the amount of sugar an animal consumed in response to saline treatment (on day 8) and the cumulated amount of dopamine overflow from the posterior-medial accumbens stimulated by amphetamine treatment, $r = 0.584$, $P < 0.05$. Fig. 2B shows that HIGH

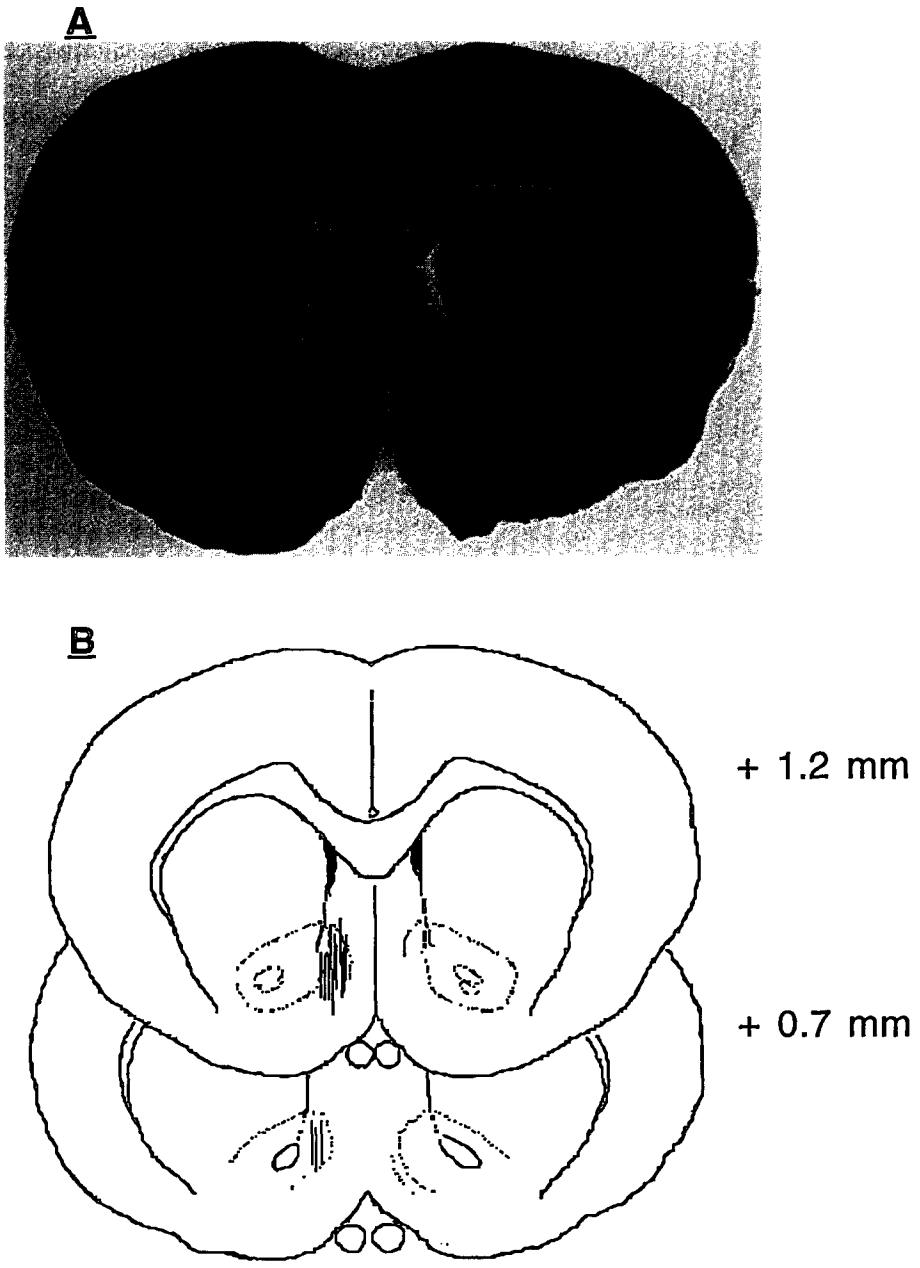

Fig. 1. A: Photomicrograph of a typical placement of a microdialysis probe within the posterior-medial nucleus accumbens (arrow points to the ventral extent of the microdialysis probe). B: Location of correct probe placements.

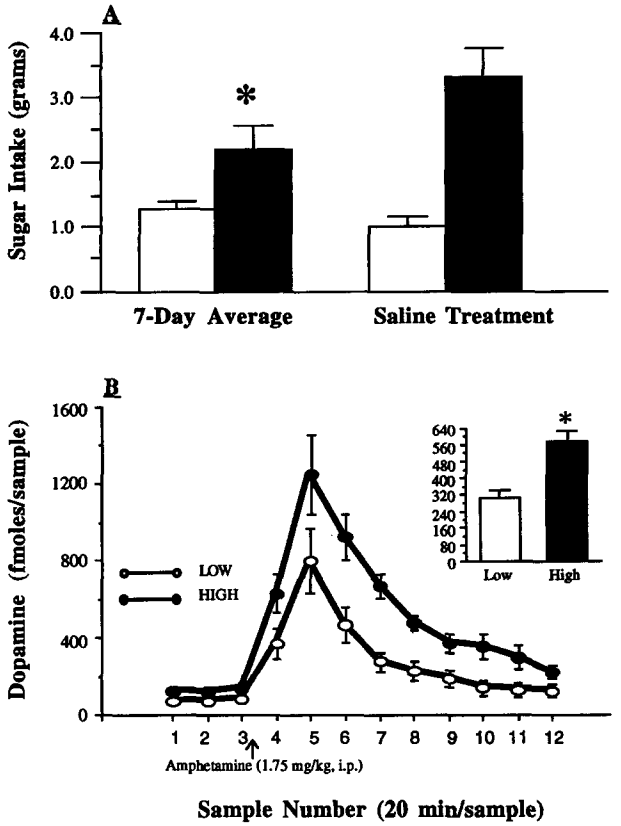

Fig. 2. A: Average amount of sugar consumed across the 7 day adaptation period and in response to saline treatment (on day 8) in animals designated as LOW (□; $n = 7$) and HIGH (■; $n = 7$) sugar feeders based on a median split of their sugar intake in response to the injection of saline ($^*P < 0.05$ as compared to LOW rats). B: Concentration of dopamine in microdialysate samples from the posterior-medial nucleus accumbens across the three baseline intervals and the nine intervals following 1.75 mg/kg (i.p.) amphetamine in animals previously designated as LOW (○; $n = 7$) and HIGH (●; $n = 7$) sugar feeders. Inset: Average concentration of dopamine per microdialysate sample in LOW (□) and HIGH (■) rats following 1.75 mg/kg (i.p.) amphetamine treatment ($^*P < 0.01$ as compared to LOW rats).

sugar feeders exhibited significantly higher levels of dopamine overflow from the posterior-medial accumbens in response to 1.75 mg/kg amphetamine than LOW sugar feeders. A two-way ANOVA, with group and intervals as the factors, revealed a significant main effect of group $F(1,12) = 12.921$, $P < 0.01$. There was also a significant main effect of intervals $F(8,96) = 28.689$, $P < 0.0005$; amphetamine treatment resulted in an immediate increase in dopamine concentration that peaked at 40 min and slowly diminished over the 3 h test session. This pattern was the same for both LOW and HIGH feeders, as indicated by the lack of a significant group × intervals interaction, $F(8,96) = 1.57$, $P > 0.05$.

Examination of dopamine concentration at baseline (the last three samples prior to amphetamine treatment) revealed that HIGH animals tended to have higher baseline dopamine levels than LOW animals, although this effect did not reach statistical significance, $t_{12} = 1.572$, $P = 0.071$. Baseline dopamine concentration for HIGH feeders was 125.9 ± 25.1 fmol/sample, while for LOW feeders it was 75.8 ± 19.8 fmol/sample.

4. Discussion

The results of the present experiment indicate that individual differences in sugar consumption predict individual differences in amphetamine-stimulated dopamine release in the posterior-medial nucleus accumbens of awake rats. There was a positive correlation between the amount of sugar consumed in response to an acute injection of saline and the cumulative amount of dopamine overflow in the posterior-medial accumbens stimulated by amphetamine. Rats that consumed high amounts of sugar (HIGH) exhibited higher levels of amphetamine-stimulated accumbens-dopamine overflow than rats that consumed low amounts of sugar (LOW).

At present the neurobiological mechanism(s) underlying the differential accumbens-dopamine response to amphetamine exhibited by LOW and HIGH sugar feeders is not known. It is interesting to speculate on alternative possibilities. LOW and HIGH sugar feeders may exhibit differences in dopamine synthesis and/or dopamine storage, and in the functioning of the dopamine transporter that may account for their differential dopamine response to amphetamine. Rats that exhibit high levels of psychostimulant-induced locomotor activity show higher cocaine-induced accumbens-dopamine overflow than rats that exhibit low levels of psychostimulant-induced locomotor activity (Hooks et al., 1992). This finding suggests that high responding animals have a larger dopamine storage pool than low responding animals since the effects of cocaine on dopamine activity are thought to be dependent on the exocytotic release of vesicular dopamine (Hurd and Ungerstedt, 1988). There is also evidence that rats with high sensitivity to the locomotor activating effects of psychostimulants have a more efficient dopamine transport mechanism that rats with low sensitivity to psychostimulant treatments (Hooks et al., 1994). Finally, rats that show high levels of psychostimulant-induced locomotor activity may also have more post-synaptic dopamine receptors than rats that show low levels of psychostimulant-induced locomotor activity (Hooks and Kalivas, 1994; Hooks et al., 1994).

In summary, the results of this experiment demonstrate that individual differences in sugar consumption predict individual differences in amphetamine-stimulated dopamine release in the posterior-medial nucleus accumbens. Previously, it has been shown that rats that exhibit a high dopaminergic response to a novel environment and to psychostimulant treatments more readily self-administer amphetamine (Piazza et al., 1989). Further, rats with high basal accumbens-dopamine turnover show higher rates of

morphine self-administration than rats with low basal accumbens-dopamine turnover (Glick et al., 1992). Thus, individual differences in the functioning of the mesolimbic dopamine system appear to underlie vulnerability to self-administer amphetamine and other drugs of abuse. One implication of the present findings is that HIGH sugar feeders may be more liable to develop drug self-administration than LOW sugar feeders. It may be the case that intrinsic variation in the functioning of the mesolimbic dopamine system is the common physiological basis for the expression of individual differences in response to natural and drug reinforcers.

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